

# Stable Mixed Hematopoietic Chimerism After Bone Marrow Transplantation for Sickle Cell Anemia

M.C. Walters,<sup>1</sup> M. Patience,<sup>1</sup> W. Leisenring,<sup>2</sup> Z.R. Rogers,<sup>3</sup> V.M. Aquino,<sup>3</sup> G.R. Buchanan,<sup>3</sup> I.A.G. Roberts,<sup>4</sup> A.M. Yeager,<sup>5</sup> L. Hsu,<sup>6</sup> T. Adamkiewicz,<sup>6</sup> J. Kurtzberg,<sup>7</sup> E. Vichinsky,<sup>1</sup> B. Storer,<sup>2</sup> R. Storb,<sup>2</sup> K.M. Sullivan<sup>1</sup> for the Multicenter Investigation of Bone Marrow Transplantation for Sickle Cell Disease (see Appendix)

<sup>1</sup>Children's Hospital Oakland, Oakland, California; <sup>2</sup>Fred Hutchinson Cancer Research Center and the University of Washington, Seattle, Washington; <sup>3</sup>University of Texas Southwestern Medical Center, Dallas, Texas; <sup>4</sup>Royal Postgraduate Medical School, London, England; <sup>5</sup>University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania; <sup>6</sup>Emory University, Atlanta, Georgia; <sup>7</sup>Duke University Medical Center, Durham, North Carolina

Correspondence and reprint requests: Mark C. Walters, MD, Children's Hospital Oakland, 747 52nd St, Oakland, CA 94609-1809 (e-mail: [mwalters@mail.cho.org](mailto:mwalters@mail.cho.org)).

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## ABSTRACT

A multicenter investigation of allogeneic bone marrow transplantation for children with sickle cell disease was conducted that included 27 European and North American transplant centers. Fifty-nine patients who ranged in age from 3.3 to 15.9 years (median, 10.1 years) received HLA-identical sibling marrow allografts between September 1991 and April 2000. Fifty-five patients survive, and 50 survive free from sickle cell disease, with a median follow-up of 42.2 months (range, 11.8 to 115 months) after transplantation. Of the 50 patients with successful allografts, 13 developed stable mixed donor-host hematopoietic chimerism. The level of donor chimerism, measured  $\geq 6$  months after transplantation in peripheral blood, varied between 90% and 99% in 8 patients. Five additional patients had a lower proportion of donor cells (range, 11% to 74%). Among these 5 patients, hemoglobin levels varied between 11.2 and 14.2 g/dL (median, 11.3 g/dL; mean, 12.0 g/dL). In patients who had donors with a normal hemoglobin genotype (Hb), the Hb S fractions were 0%, 0%, and 7%, corresponding to donor chimerism levels of 67%, 74%, and 11%, respectively. Among patients who had donors with sickle trait, the Hb S fractions were 36% and 37%, corresponding to donor chimerism levels of 25% and 60%, respectively. Thus, allograft recipients with stable mixed chimerism had Hb S levels similar to donor levels, and only 1 patient required a red blood cell transfusion beyond 90 days posttransplantation. None of the patients have experienced painful events or other clinical complications related to sickle cell disease after transplantation. These observations strongly suggest that patients with sickle cell disease who develop persistent mixed hematopoietic chimerism after transplantation experience a significant ameliorative effect.

## KEY WORDS

Chimerism • Bone marrow transplantation • Sickle cell anemia

## INTRODUCTION

The primary objective of hematopoietic cell transplantation (HCT) in the treatment of hematological disorders is to eradicate the underlying disorder and replace defective host cells with donor hematopoietic cells. Traditionally, this objective has been achieved by myeloablative therapy before HCT and subsequent recovery of donor hematopoiesis, resulting in full donor chimerism. With the development of more sensitive means to detect residual host cells, it became possible to study the impact of mixed donor-host hemato-

poietic chimerism after HCT on outcomes such as engraftment, graft-versus-host disease (GVHD), and disease recurrence [1,2]. In the setting of malignant disorders, the emergence of host hematopoietic cells after HCT was correlated with the delivery of less-intensive pretransplantation conditioning therapy and with T-cell depletion of donor grafts [3,4]. Those patients who developed mixed chimerism benefited from a decreased risk of GVHD [4-7]. Although mixed chimerism was not universally predictive of disease recurrence [5,8-10], abrogation of the graft-versus-leukemia

(GVL) activity with donor-host T-cell chimerism was predictive of relapse in certain clinical settings (eg, T cell-depleted transplantation for chronic myelogenous leukemia) where the GVL effect remains an important factor for eliminating minimal residual disease [6,11,12]. Thus, the benefits of residual host hematopoiesis and lymphoid chimerism after HCT for malignant disorders remain somewhat uncertain.

In contrast, among those patients who undergo HCT for nonmalignant disorders, the development of stable mixed donor-host hematopoietic chimerism has the potential for considerable ameliorative effect, an observation that has been particularly well documented for  $\beta$ -thalassemia major and other hereditary disorders [13-17]. The principal negative consequence of developing mixed lymphohematopoietic chimerism after HCT for these disorders is graft rejection, usually accompanied by disease recurrence, typically occurring in the first year after HCT [15,18]. However, when stable hematopoietic chimerism develops after HCT, it appears that even a minority of donor cells is sufficient to overcome an underlying genetic defect. In the example of thalassemia major, there is an apparent enrichment of donor erythrocytes in the blood, presumably by virtue of their improved survival compared to their host counterparts, which are destroyed during ineffective erythropoiesis.

Here we update and extend the observations of stable mixed donor-host chimerism after conventional myeloablative transplantation for sickle cell anemia. In this international, multicenter, prospective clinical investigation, we confirm that mixed donor-host chimerism after HCT for hemoglobin disorders has significant therapeutic benefit.

## PATIENTS AND METHODS

### Patients

Patients younger than 16 years who had symptomatic sickle cell disease (SS-, SC-, or S $\beta$ -thalassemia) and who had HLA-identical family member donors (Hb AA or Hb AS) were considered for marrow transplantation. All individuals were required to meet eligibility criteria as reported earlier [19]. Patients with extensive end-organ dysfunction were excluded from enrollment. These conditions included significant functional impairment (as determined by the Lansky Play Scale or a Karnofsky score <70%), hepatic disease (active hepatitis or cirrhosis), severe renal impairment (glomerular filtration rate, <30% predicted normal for age), severe residual functional neurologic impairment (hemiplegia alone was not an exclusion), or stage III-IV sickle cell lung disease. Patients were enrolled from 27 centers in the United States and Europe (see the "Appendix" for collaborating centers). The study was approved by the institutional review board of the Fred Hutchinson Cancer Research Center and by institutional review boards or their equivalents at each of the collaborating sites. All patients and/or their parents or guardians gave written informed consent for their participation.

The National Heart, Lung, and Blood Institute appointed a data safety and monitoring board (DSMB) to monitor patient safety and the ethical conduct and progress of this investigation. The board consisted of 5 hematologists, a clinical statistician, and a patient advocate. The

principal investigators (M.C.W. and K.M.S.) submitted quarterly reports to the DSMB.

### Treatment Regimen

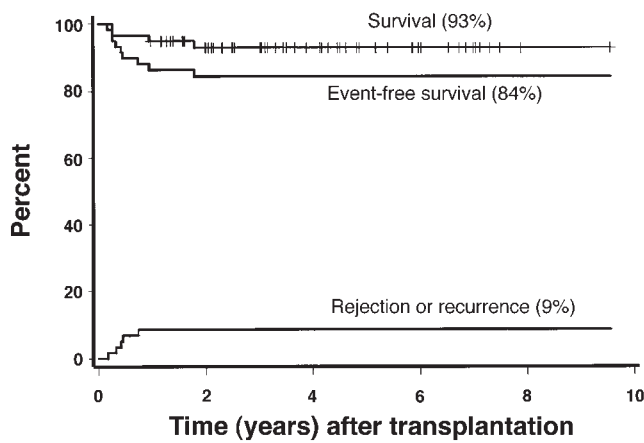
Conditioning treatment to prepare patients for transplantation consisted of a combination of busulfan (BU, 14 mg/kg), cyclophosphamide (CY, 200 mg/kg), and horse antithymocyte globulin (ATGAM, 90 mg/kg; Pharmacia and UpJohn Co, Peapack NJ), or CAMPATH immunoglobulin (10 mg for 5 days) in lieu of ATG [19]. Three patients (patients 9, 10, and 11) received BU (500 mg/m<sup>2</sup>), CY (200 mg/kg), and rabbit ATG (20 mg/kg), and another patient (patient 6) received BU (16 mg/kg), CY (200 mg/kg), and ATG (80 mg/kg). Since November 1994, all North American patients had BU pharmacokinetics performed with targeted steady-state concentrations adjusted to 400-600 ng/mL. Patients received a combination of methotrexate and cyclosporine (CSP) for the prevention of acute GVHD as previously reported [20,21]. CSP prophylaxis was given for 6 months following transplantation. Definition and grading of acute and chronic GVHD have been described [20,21].

### Supportive Care

Prior to transplantation, patients not receiving chronic transfusion therapy underwent partial exchange transfusions to achieve fractions of Hb S that were  $\leq$ 30%. All patients who were seronegative for cytomegalovirus (CMV) received CMV-antibody-negative screened blood products. Patients also received prophylactic intravenous broad-spectrum antibiotics after transplantation and oral penicillin for at least 2 years or longer until splenic recovery was documented by liver-spleen radionuclide scans after transplantation. In response to an apparent increased incidence of neurologic complications after transplantation [22], the following guidelines were employed since June 1993: anticonvulsant prophylaxis with phenytoin initiated with BU dosing and continued for 6 months following transplantation (or until CSP was discontinued); strict control of hypertension; prompt repletion of magnesium deficiency; and maintenance of hemoglobin concentrations of 9 to 11 g/dL and platelet counts of >50,000/ $\mu$ L.

### Analyses of Chimerism

Identification of hematopoietic cell chimerism was made by Y-body in situ hybridization of cells from peripheral blood mononuclear cells when donor and recipient were sex-mismatched. When donor-host pairs were not sex mismatched, chimerism analysis was performed by Southern blotting of variable-number tandem repeats (VNTR) in peripheral blood genomic DNA using the YNH, M27 $\beta$ , or TBQ probes, by restriction fragment length polymorphism (RFLP), or by microsatellite analysis. To assess the relative contributions of donor and recipient, these assays were quantitated by densitometry of autoradiographs for VNTR and RFLP analyses. Assay sensitivities to 5% for these techniques were confirmed by mixing experiments of known amounts of patient and donor cells or DNA. These analyses were performed at day 84 and at 6 months following transplantation, and biannually thereafter if evidence of mixed chimerism was present. Microsatellite analyses were



**Figure 1.** Outcome after transplantation for 59 children with advanced symptomatic sickle cell disease. Kaplan-Meier estimates for survival and event-free survival following marrow transplantation are shown. An event is defined as death, graft rejection, or recurrence of sickle cell disease. A cumulative incidence curve for graft rejection and return of sickle cell disease is also depicted.

performed using standard techniques [23,24]. These studies were performed in parallel with evaluations of hemoglobin levels and Hb S fraction quantification by electrophoresis.

#### Late Effects Evaluation

Cerebral magnetic resonance imaging (MRI) and angiography (MRA) examinations were requested for all patients before, and 1 and 2 years after, transplantation. Similarly, pulmonary function tests (total lung capacity, forced vital capacity [FVC], residual volume, and the ratio of forced expiratory volume to FVC) were measured before and at annual intervals after transplantation.

Pulmonary function testing was based on common methods for comparison of reference values [25]. Actual lung volumes were compared to predicted values from age- and sex-matched control subjects to generate a percentage of the predicted value for each subject. The staging criteria for sickle cell pulmonary disease described by Powars et al. was used for grading restrictive pulmonary disease [26]. Mild restrictive pulmonary disease was defined as 80% of predicted normal or 1 standard deviation (SD) below normal; moderate was defined as 60% of predicted normal, or

2 SDs below normal; and severe was defined as 40% of predicted normal, or 3 SDs below normal.

#### Statistical Analysis

Statistical analyses were performed to summarize results. The method of Kaplan and Meier was used to estimate survival and event-free survival (where an event was defined as death, graft rejection, or return of sickle cell disease) [27]. A cumulative-incidence curve for graft rejection was also calculated [28]. Event-free survival was defined as survival in the absence of clinical vaso-occlusive complications typical of sickle cell disease. Stable mixed chimerism was defined as 5% to 95% donor chimerism, as determined by standard molecular techniques, 6 months or longer after transplantation.

Hazard ratios were estimated from proportional hazards regression models. Death without rejection was treated as a competing-risk event, and patients were censored at the time of death. All *P* values were 2-sided and were based on likelihood-ratio statistics.

#### RESULTS

Fifty-nine children with sickle cell anemia, sickle  $\beta^+$ -thalassemia, or sickle- $O^{\text{Arab}}$  received HLA-identical sibling marrow allografts between September 1991 and April 2000. Of these patients, 50 survive free of disease with a median follow-up of 42.2 months (range, 11.8 to 115 months). Five patients had graft rejection followed by return of sickle cell disease a median of 5.1 months (range, 2.1 to 8.9 months) after HCT (Table 3), and 4 patients died of intracranial hemorrhage (*n* = 1) or GVHD (*n* = 3). The Kaplan-Meier probabilities of survival and event-free survival were 93% and 84%, respectively (Figure 1). The cumulative incidence of recurrent sickle cell disease was 9%. Among the 50 patients surviving free of sickle cell disease, all but 1 had chimerism studies performed after HCT. Of these, 13 patients (9 male, 4 female) had stable mixed donor-host hematopoietic chimerism detected after HCT. For this analysis, patients with stable mixed chimerism were divided into 2 groups. Group 1 consisted of patients with 90% or greater donor chimerism (*n* = 8), and group 2 had patients with <75% donor cells after HCT (*n* = 5).

#### Characteristics of Patients With Mixed Chimerism

Thirteen patients with stable mixed chimerism ranged in age from 4.2 to 13 years (median, 6.9 years). The indications for transplantation are shown in Tables 1 and 2. Seven

**Table 1.** Characteristics of Patients Who Developed Stable Mixed Chimerism With  $\geq 90\%$  Donor Chimerism\*

Patient	Age at BMT, y	Indication for BMT	Donor Hb Genotype	Time After BMT, mo	Percentage Donor Chimerism, %	Hb S, %
22	6.9	Recurrent VOC	AA	48.2	90-95	—
42	7	Stroke	AS	12.2	95-99	40
43	4	CNS disease	AS	7.8	90	34
44	12	Recurrent ACS	AS	14.3	95	41
45	9	Recurrent ACS	AS	13	90-95	38
46	4	Recurrent ACS	AA	9	93	0
50	7.6	Stroke	AS	12.3	90	37
55	6	Stroke	AS	17	90	38

\*VOC indicates vaso-occlusive crises; AA, healthy donor; AS, sickle cell-trait donor; ACS, acute chest syndrome.

**Table 2.** Characteristics of Patients Who Developed Stable Mixed Chimerism With <75% Donor Chimerism

Patient	Age at BMT, y	Indication for BMT	Donor Hb Genotype	Time After BMT, mo	Percentage Donor Chimerism, %	Hemoglobin Concentration, g/dL	Hb S, %	Retic, %*
13	5.2	Stroke	AA	64	11	11.3	7	7.8†
18	8.2	Recurrent ACS	AA	70	67	14.2	0	1.0
38	5.4	Stroke	AS	36	20-30	11.8	36	1.9
49	5	Stroke	AA	29	74	11.3	0	—
52	5	Recurrent ACS	AS	22	60	11.2	37	1.5

\*Retic indicates reticulocyte production index.

†Uncorrected reticulocyte count.

patients had a stroke or other significant central nervous system (CNS) disease before HCT, 5 had recurrent episodes of acute chest syndrome, and 1 had recurrent vaso-occlusive painful crises. Thus, the distribution of eligibility criteria among those with stable mixed chimerism did not differ significantly from the larger group of 59 patients. Donor hemoglobin genotype is shown, and 7 patients had donors with sickle cell trait. Six patients had received more than 10 red blood cell (RBC) transfusion exposures before HCT, 5 received fewer than 10, and, in 2 patients, the transfusion exposure before HCT was not known. Serum ferritin levels varied between 58 and 5066 ng/mL (median, 830 ng/mL). Three patients had evidence of alloimmunization to RBC antigens before HCT. Platelet antibody screening was performed in 8 patients before HCT, and results were negative in each patient tested. Eight of 13 patients had busulfan pharmacokinetics tests performed to ensure average steady-state levels of 400 to 600 ng/mL in the blood. Three of 5 patients in group 2 had steady-state levels of 413, 450, and 619 ng/mL; in 2 patients, pharmacokinetics studies were not performed. There was no major ABO blood type incompatibility among group 2 donor-host pairs. ABO incompatibility was found in 1 pair among 8 group 1 patients. All patients received bone marrow allografts with a median cell dose (of total nucleated cells [TNC]) of  $3.0 \times 10^8$ /kg recipient weight (range,  $0.82$ - $5 \times 10^8$ /kg recipient weight). The median doses of TNC infused among group 1 and group 2 patients who developed stable mixed chimerism were  $2.4 \times 10^8$ /kg and  $3.1 \times 10^8$ /kg, respectively.

### Impact of Mixed Chimerism on Outcome

Overall, 11 of 59 patients (19%) developed acute or chronic GVHD, which was the cause of death in 3 patients. In contrast, none of 13 patients with stable mixed chimerism experienced acute or chronic GVHD ( $P = .05$ ). There was no apparent association between the development of mixed chimerism and graft rejection/disease recurrence. This observation contrasts with observations after transplantation for cases of  $\beta$ -thalassemia major, in which mixed chimerism was associated with an increased risk of disease recurrence [14]. Other factors evaluated for their association with developing stable mixed chimerism and rejection after transplantation for sickle cell disease included patient age, patient sex, donor sex, donor hemoglobin genotype, the number of transfusions received, incidence of RBC alloimmunization, and the presence or absence of chelation therapy. A proportional hazards regression analysis was performed to determine if any of these factors was associated with recurrent

disease or with developing stable mixed chimerism, and the results are presented in Tables 3 and 4, respectively. Although most factors failed to achieve statistical significance, in part because of the infrequency of these events, there was a trend suggesting that patients receiving chelation therapy for transfusional iron overload had an increased risk of recurrent sickle cell disease ( $P = .04$ ). Of interest, neither RBC transfusion exposures or iron chelation therapy was associated with developing stable mixed chimerism after transplantation. However, patients younger than 10 years were significantly more likely to develop mixed chimerism than those who were older than 10 years ( $P = .001$ ) (Table 4).

To determine the impact of stable mixed chimerism on sickle cell disease expression, group 2 patients were monitored for hematological and clinical manifestations of the underlying disease (Table 2). These 5 patients had stable mixed chimerism with a follow-up period that ranged from 22 to 70.2 months (median, 36.3 months) after HCT, when last evaluated. Hemoglobin levels varied from 11.2 to 14.2 g/dL (median, 11.3 g/dL). Only patient 38 required a

**Table 3.** Univariate Analysis of Risks for Graft Rejection/Recurrent Disease After Transplantation for Sickle Cell Disease\*

Risk Factor	No. of Patients With Graft Rejections	OR (95% CI)	P
<b>Age at transplantation</b>			
<10 y (n = 29)	2	1.0	.71
≥10 y (n = 30)	3	1.4 (0.2-8.4)	
<b>Patient sex</b>			
Female (n = 23)	3	1.0	.38
Male (n = 36)	2	0.5 (0.1-2.7)	
<b>Donor sex</b>			
Female (n = 29)	4	1.0	.18
Male (n = 25)	1	0.3 (0.0-2.3)	
<b>Donor genotype</b>			
AS (n = 35)	3	1.0	.94
AA (n = 23)	2	0.9 (0.2-5.6)	
<b>RBC alloimmunization</b>			
No (n = 40)	3	1.0	.53
Yes (n = 16)	2	1.8 (0.3-11)	
<b>Chelation therapy</b>			
No (n = 36)	1	1.0	.04
Yes (n = 21)	4	7.3 (0.8-65)	
<b>Pre-BMT ferritin levels, ng/mL</b>			
<2000 (n = 43)	3	1.0	.93
≥2000 (n = 12)	1	1.1 (0.1-11)	

\*OR indicates odds ratio; CI, confidence interval.



RBC transfusion more than 90 days post-HCT, and this patient currently remains independent of RBC transfusions. Reticulocyte fractions were elevated in 2 patients who had the lowest levels of donor chimerism (Table 2, patients 13 and 38). The Hb S fractions, depicted serially in Figures 1 and 2, demonstrated that Hb S fractions closely matched donor Hb S levels, even when there was a minority of donor cells. Together, these data strongly suggest that there was an enrichment of donor RBCs due to their longer life span.

Clinically, among all group 1 and 2 patients, there were no painful vaso-occlusive crises after HCT, and no patients experienced other sickle cell-related clinical complications. There were no significant differences in hematological or clinical observations when recipients who had sickle cell trait and donors of normal hemoglobin were compared.

Group 2 patients were also monitored for effect of HCT on pulmonary function and on cerebral imaging by MRI as previously reported (Table 5). These evaluations showed stabilization of abnormalities that were present before HCT. As previously reported, patient 18, who had been studied by cerebral MRI 3 months before transplantation, developed new MRI lesions 1 month after transplantation, but subsequent annual studies have remained stable. It is likely that this patient had progression of CNS disease before transplantation. All 13 group 1 and 2 patients with stable mixed donor host chimerism were screened for quality of life, and all have Karnofsky or Lansky Play scale scores of 100%. Thus, together, these data demonstrate that patients who develop stable mixed chimerism after HCT experience clinical and subclinical cessation of sickle cell-related events with excellent quality of life and no chronic GVHD.

## DISCUSSION

This report updates observations of stable mixed donor-host hematopoietic chimerism that developed after conventional myeloablative HCT for children with symptomatic sickle cell disease. These observations mirror similar phenomena after allografting for  $\beta$ -thalassemia major, in which up to 10% of patients had residual host cells that persisted for longer than 2 years after HCT [13,14,17,29]. These results are also similar to observations by European centers after conventional HCT for sickle cell disease, where 12 of 103 patients developed stable mixed chimerism [30-33]. Although our results are not entirely novel, a systematic evaluation of the outcome of stable mixed chimerism in sickle cell disease has not previously been performed, and thus these observations provide a rational basis for considering nonmyeloablative HCT for hemoglobinopathies. In both hemoglobin disorders, there were no apparent deleterious effects of residual erythropoiesis by defective host cells, an outcome consistent with the idea that replacement by full donor chimerism is not a requirement for significant amelioration, if not cure, of these disorders. Although a longer period of follow-up will be required to assess the long-term impact of mixed chimerism on the clinical and subclinical expression of sickle cell disease, it is possible that, similar to chronic RBC transfusions, mixed chimerism will significantly reduce but not altogether eliminate adverse clinical events such as stroke [34,35]. Alternatively, the experience of mixed chimerism after transplantation for leukocyte-

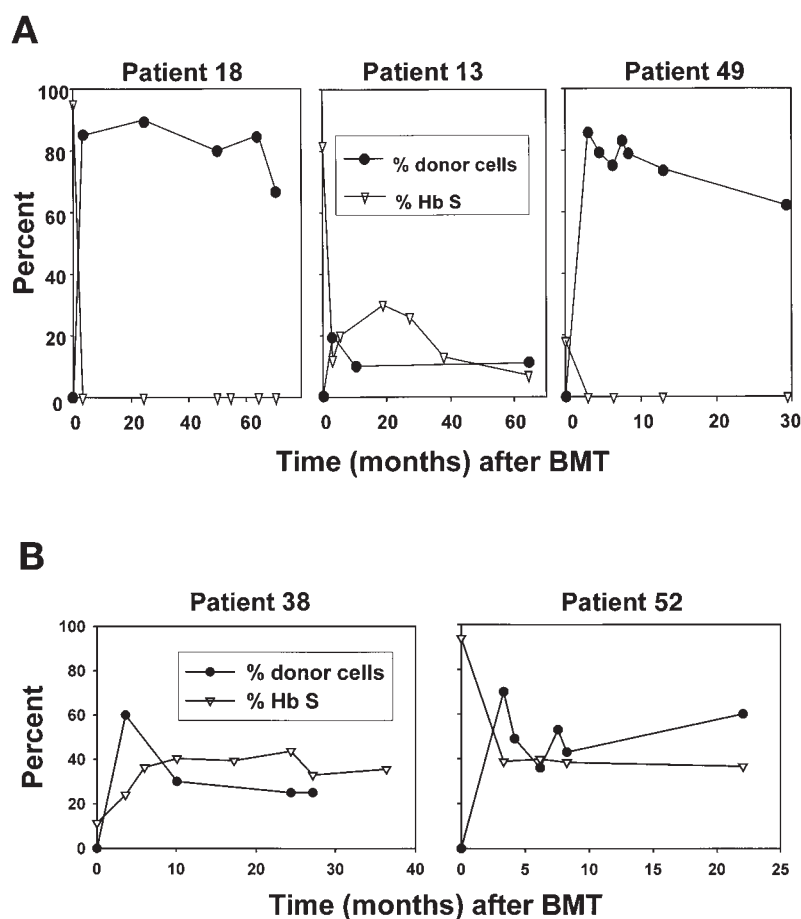
**Table 4.** Univariate Analysis of Risks for Developing Stable Mixed Chimerism (MC) After Transplantation for Sickle Cell Disease

Risk Factor	No. of Patients With Stable MC*	OR (95% CI)	P
<b>Age at transplantation</b>			
<10 y (n = 28)	12	1.0	
≥10 y (n = 29)	2	0.1 (0.02-0.5)	.001
<b>Patient sex</b>			
Female (n = 22)	5	1.0	
Male (n = 35)	9	1.2 (0.3-4.1)	.80
<b>Donor sex</b>			
Female (n = 28)	7	1.0	
Male (n = 25)	7	1.2 (0.3-4.0)	.80
<b>Donor genotype</b>			
AS (n = 33)	8	1.0	
AA (n = 23)	6	1.1 (0.3-3.8)	.88
<b>RBC alloimmunization</b>			
No (n = 40)	9	1.0	
Yes (n = 14)	4	1.4 (0.4-5.5)	.65
<b>Chelation therapy</b>			
No (n = 35)	10	1.0	
Yes (n = 20)	3	0.4 (0.1-1.8)	.24
<b>Pre-BMT ferritin levels, ng/mL</b>			
<2000 (n = 41)	11	1.0	
≥2000 (n = 12)	3	0.9 (0.2-4.0)	.90

\*One rejecting patient also had MC >6 months after transplantation.

adhesion defect [16], selected metabolic storage disorders [36], immunodeficiency syndromes [37], and chronic granulomatous disease [38-40] supports the idea that low-level (15% to 30%) donor chimerism is sufficient for a significant beneficial effect in these hereditary disorders. It is likely that among those who have very low-level donor chimerism after transplantation for sickle cell anemia, the clinical benefit will hinge on the extent to which circulating sickle erythrocytes are reduced or eliminated [41]. However, in our small cohort, we found no evidence that those with a minority of donor cells experienced exacerbation of their disease, in contrast to observations of chimerism after transplantation in a murine model of sickle cell anemia [42].

The natural enrichment of donor erythrocytes is perhaps not very surprising in  $\beta$ -thalassemia major, a hemoglobin disorder in which anemia is largely the consequence of ineffective erythropoiesis [43]. In this setting, normal erythroid precursors should have a survival advantage over thalassemic counterparts in the marrow and thus generate a majority of donor erythrocytes in the blood. In sickle cell anemia, however, peripheral hemolysis is a key determinant of anemia. Nonetheless, here too we observed a survival advantage of donor erythrocytes, and even a small fraction of donor cells eliminated clinical and subclinical evidence of vaso-occlusion, although some patients had a persistent mild anemia. Previously, we reported overrepresentation of donor erythroid progenitors in the marrow compared to donor myeloid counterparts in a patient who evolved to develop graft rejection [44], observations that were recapitulated in patients who developed mixed chimerism after nonmyeloablative preparation for transplantation [45]. Together, these observations suggest that donor erythroid progenitors have a survival advantage over sickle counterparts in the marrow. Thus, efforts to repopulate hemoglobinopathy



**Figure 2.** Serial determinations of donor chimerism and Hb S fractions after transplantation. A, The fraction of donor cells in the blood (●) and Hb S fraction (▽) are depicted at regular intervals after transplantation with a follow-up period extending to more than 5 years after transplantation. The relationship between donor chimerism and the Hb S fraction is shown. Patients 13, 18, and 49 who had Hb AA donors are shown. B, Hb S and chimerism studies from patients 38 and 52 who had Hb AS donors are shown.

recipients with normal erythrocytes that have a natural survival advantage in the marrow and in circulation might hold the key to success in curing these disorders. The source of these cells might be autologous hematopoietic stem cells transduced by a replacement gene vector that is able to produce stable levels of a developmentally regulated  $\beta$ -globin gene over repetitive cell cycles, at a balanced level in relation to the complementary  $\alpha$ -globin chain [44]. Given the complexities of accomplishing this aim, a more expedient alternative might be to use HLA-compatible donors after preparation for transplantation with nonmyeloablative but immunosuppressive therapy to establish stable hematopoietic chimerism [46,47]. Here the principal challenge is to overcome immunological barriers to donor-host tolerance, barriers that were overcome serendipitously after conventional myeloablative conditioning in the cohort we described here. To explore this challenge, we have initiated a multicenter collaborative investigation of nonmyeloablative preparation before allogeneic marrow transplantation for symptomatic sickle cell disease.

The possibility of inducing stable mixed hematopoietic donor-host chimerism presents several potential advantages over treatment with conventional conditioning regimens.

First, by using less toxic conditioning regimens, the resultant tissue injury caused by cytoreductive therapy might be mitigated. In addition, it is possible that conditions conducive to cytokine secretion and host peptide fragment presentation that are postulated to promote GVH reactions might be decreased as a result of eliminating the administration of high-dose chemotherapy and radiation before HCT [48-50]. Finally, a steady source of host lymphocytes and antigen-presenting cells (APC) that survive nonmyeloablative conditioning might promote donor-host tolerance by negative selection of alloreactive donor T cells in the thymus and peripheral locations [51]. Thus, the risk of GVHD in those patients who develop stable chimerism might be reduced or even eliminated after HCT using cells from HLA-identical sibling donors. Concerns that concurrent major histocompatibility complex restriction by host thymocytes and by donor APC might actually induce a state of immunodeficiency have not been realized. In fact, immune reconstitution appears accelerated among those who develop stable chimerism [52,53]. In animals that developed stable mixed chimerism, immune-competent grafts were established that supported donor-specific tolerance, as indicated by the acceptance of donor skin and organ grafts [54,55].

**Table 5.** Follow-up Studies of Cerebral Imaging and Pulmonary Function Among Group 2 Patients With Stable Mixed Donor-Host Chimerism\*

Patient No.	Indication for BMT	Baseline Cerebral MRI	1 Year Post-BMT	3 and ≥4 Years Post-BMT		
13	Stroke	Multiple periventricular and R. frontal foci of increased T2 signal		Stable		
18	ACS	Leukomalacia	New R. frontal-parietal infarct	Stable/stable		
38	Stroke	R. MCA infarction/R. brainstem abnormality	Stable			
49	Stroke	L. frontal and bilateral corona radiata and centrum semiovale abnormalities	Stable			
52	Recurrent ACS	Bilateral fronto-parietal foci with high signal	Stable			
Patient No.	Indication for BMT	Baseline PFT Results	1 Year Post-BMT	2 Years Post-BMT	3 Years Post-BMT	≥4 Years Post-BMT
13	Stroke	Normal				
18	ACS, abnormal MRI	Mild RPD	Mild RPD	Mild RPD	Mild to moderate RPD	Mild RPD
38	Stroke	Moderate RPD		Moderate RPD		
49	Stroke	Not studied (too young)				
52	Recurrent ACS	Normal	Normal			

\*R. indicates right; MCA, middle carotid artery; L., left; PFT, pulmonary function test; RPD, restrictive pulmonary disease.

Thus, nonmyeloablative regimens aimed at inducing mixed chimerism are particularly attractive in the setting of hereditary anemias such as sickle cell anemia, where the chief concerns of extending the option of stem cell transplantation to all patients are the risks of adverse events associated with conventional cytoreductive therapy and GVHD. Logically, older patients who have experienced chronic vaso-occlusive organ damage that excludes them from conventional allogeneic transplantation might make ideal candidates for an initial clinical trial. However, transfusion exposures that increase the risk of graft rejection [56] and GVHD in older recipients should prompt caution until a preparative regimen to control HVG and GVH reactions in these individuals has been defined. The results of this study support the notion that younger patients might be more likely than adults to develop stable mixed chimerism after transplantation. First, we found that graft rejection after conventional transplantation was associated with iron chelation therapy. It is possible that chelation therapy is a surrogate for exposures to minor histocompatibility antigens expressed on leukocytes in RBC products, which generated alloreactive immune responses responsible for graft rejection. Thus, younger patients with fewer transfusion exposures before transplantation might experience a lower probability of disease recurrence after nonmyeloablative preparation. Second, we observed an association between younger age and having stable mixed chimerism after conventional transplantation. Thus, it is possible that preserved thymic activity in these younger patients was a determinant that promoted bidirectional donor-host tolerance. In particular, a lower rate of GVHD in children is very likely to contribute to a better outcome, a prediction supported in part by recent observations after nonmyeloablative transplantation in 2 adult patients with sickle cell disease [57]. Still,

given the attendant risk of graft rejection that is very likely to follow nonmyeloablative preparation, it will be important to study this approach in the setting of a well-controlled multicenter clinical investigation by centers experienced in allogeneic transplantation. If successful, however, this novel therapy would dramatically alter the approach to transplantation for hereditary anemias like sickle cell disease and extend its availability.

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## APPENDIX

The following investigators and centers participated in this collaborative study: *Ann Arbor, MI* (J. Levine, University of Michigan); *Atlanta, GA* (J.R. Eckman, L. Hsu, T. Adamkiewicz, A. Yeager, Emory University); *Birmingham, UK* (P.J. Darbyshire); *Boston, MA* (E. Guinan, O. Platt, Dana Farber Cancer Institute and The Children's Hospital, Harvard University; L. McMahon, Boston Comprehensive Sickle Cell Center); *Bronx, NY* (L. Benjamin, R. Nagel, Montefiore Medical Center, Albert Einstein College of Medicine); *Brooklyn, NY* (L. Guarini, Interfaith Medical Center; K. Viswanathan, Brookdale Hospital; Rita Bellevue, NY Methodist Hospital); *Chapel Hill, NC* (R. Redding-Lallinger,

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